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Phylogeny is a powerful tool for predicting plant biomass responses to nitrogen enrichment

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Abstract. Increasing rates of anthropogenic nitrogen (N) enrichment to soils often lead to the dominance of nitrophilic plant species and reduce plant diversity in natural ecosystems. Yet, we lack a framework to predict which species will be winners or losers in soil N enrichment scenarios, a framework that current literature suggests should integrate plant phylogeny, functional tradeoffs, and nutrient co-limitation. Using a controlled fertilization experiment, we quantified biomass responses to N enrichment for 23 forest tree species within the genus *Eucalyptus* that are native to Tasmania, Australia. Based on previous work with these species' responses to global change factors and theory on the evolution of plant resource-use strategies, we hypothesized that (1) growth responses to N enrichment are phylogenetically structured, (2) species with more resource-acquisitive functional traits have greater growth responses to N enrichment, and (3) phosphorus (P) limits growth responses to N enrichment differentially across species, wherein P enrichment increases growth responses to N enrichment more in some species than others. We built a hierarchical Bayesian model estimating effects of functional traits (specific leaf area, specific stem density, and specific root length) and P fertilization on species' biomass responses to N, which we then compared between lineages to determine whether phylogeny explains variation in responses to N. In concordance with literature on N limitation, a majority of species responded strongly and positively to N enrichment. Mean responses ranged three-fold, from 6.21 (*E. pulchella*) to 16.87 (*E. delegatensis*) percent increases in biomass per g $N{\cdot}m^{-2}{\cdot}yr^{-1}$ added. We identified a strong difference in responses to N between two phylogenetic lineages in the *Eucalyptus* subgenus *Symphyomyrtus*, suggesting that shared ancestry explains variation in N limitation. However, our model indicated that after controlling for phylogenetic non-independence, eucalypt responses to N were not associated with functional traits (although post-hoc analyses show a phylogenetic pattern in specific root length similar to that of responses to N), nor were responses differentially limited by P. Overall, our model results suggest that phylogeny is a powerful predictor of winners and losers in anthropogenic N enrichment scenarios in Tasmanian eucalypts, which may have implications for other species.

Key words: biomass; Eucalyptus; *evolutionary history; functional traits; global change; hierarchical Bayesian model; nitrogen enrichment; nutrient co-limitation; phosphorus; phylogeny; plant economics spectrum; resource-use tradeoff.*

Introduction

Nitrogen (N) is the predominantly limiting nutrient to net primary productivity across terrestrial ecosystems (Vitousek and Howarth 1991, LeBauer and Treseder 2008); however, N does not limit biomass production equally across terrestrial plant species (Bobbink et al. 2010, De Schrijver et al. 2011). For example, Wooliver et al. (2016) found that across 125 terrestrial plant species, average total biomass responses to N enrichment range from −6% to 187% for each gram of $N{\cdot}m^{-2}{\cdot}yr^{-1}$ added. Such variation in N limitation explains why increased rates of N inputs to plant communities often lead to greater community productivity but lower species richness due to competitive exclusion of less nitrophilic species (Bobbink et al. 2010). This downward trajectory in plant diversity should continue because fossil fuel combustion and agricultural fertilization are predicted to more than double the rates of anthropogenic N enrichment to soils by the end of the century (Lamarque et al. 2005). Because plant diversity has consequences for above- and belowground biodiversity as well as ecosystem functioning (Zak et al. 2003, Whitham et al. 2006), it is important to determine possible factors associated with variation in plant species' performances under N enrichment scenarios.

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Phylogeny may be a predictor of plant responses to N enrichment. Specifically, phylogenetic analyses have shown that plant species' responses to global change are more similar among close relatives than would be expected at random (Edwards et al. 2007, Davis et al. 2010, Becklin et al. 2014, Wooliver et al. 2016). These patterns are likely reflections of plant functional traits that are important in resource use and show phylogenetic signal (Cavender-Bares et al. 2009, Cornwell et al. 2014), suggesting that phylogenies can be used to predict plant winners and losers in N enrichment scenarios. However, phylogenetic patterns in plant responses to N enrichment do not always persist at smaller phylogenetic scales (i.e., groups of more closely related, co-occurring species; Bennett and Cahill 2013), because of greater divergence or convergence of functional traits towards the tips of phylogenies (Ackerly 2004, Cavender-Bares et al. 2009, Cadotte et al. 2013). For example, Cavender-Bares et al. (2004) found that resource-use traits such as shoot transpiration are evolutionarily convergent in a group of 17 oak species that co-occur in North Central Florida, USA. Therefore, functional traits that influence resource-use strategies may serve as better predictors of winners and losers in N enrichment scenarios than phylogeny.

Decades of theoretical and experimental work suggest that plant functional traits directly influence capacities for resource acquisition and use: an outcome of an evolutionary tradeoff between resource-acquisitive and resource-conservative growth strategies (Grime 1977, Chapin 1980, Wright et al. 2004, Craine 2009, Reich 2014). According to this theory—termed the plant economics spectrum—species' functional traits (specifically, those affecting resource uptake, transport, and processing capacities; Violle et al. 2007) will evolve to be resource-conservative (low capacity for resource use) in low-resource environments and resource-acquisitive (high capacity for resource use) in high-resource environments as a result of the return on investment in resource acquisition. For example, empirical work has shown that plants occurring on infertile soils have lower-quality foliage, slower photosynthetic and growth rates, and lower requirements for soil nutrients (Grime 1977, Hobbie 1992, Cavender-Bares et al. 2004). Alternatively, species found on fertile soils have higher specific leaf area and faster photosynthetic rates, which accommodate higher growth rates (Reich et al. 1997, Cavender-Bares et al. 2004, Poorter and Bongers 2006, Wright et al. 2010). It might intuitively follow that species with more resource-acquisitive functional traits should exhibit greater biomass responses to N enrichment than species with more resource-conservative functional traits. However, we are unaware of any study that has investigated the associations between plant functional traits and N limitation, especially at smaller phylogenetic scales.

Co-limitation by phosphorus (P) may also govern plant biomass responses to increased soil N (Elser et al. 2007, Harpole et al. 2011). Co-limitation occurs when biomass responses to one nutrient (e.g., N) increase with the addition of another nutrient (e.g., P) (Harpole et al. 2011). Although global anthropogenic P inputs to the biosphere are now four times greater than that of natural chemical weathering (Falkowski et al. 2000), they are generally not sufficient to relieve P limitation under anthropogenic N deposition scenarios (Vitousek et al. 2010). Species' responses to N should be differentially co-limited by P if species have evolved different solutions to deal with co-limitation by P such as increased P use efficiency or the ability to scavenge for P through associations with fungal symbionts (Cleland and Harpole 2010). In this case, species whose responses to N are less limited by P (expected winners in N enrichment scenarios) should show increases in productivity and abundance over more strongly co-limited species (expected losers in N enrichment scenarios).

Using a group of 23 *Eucalyptus* tree species that are native to Tasmania, Australia, whose responses to global change factors have been shown to be phylogenetically structured (Senior et al. 2013, Wooliver et al. 2014), we examined whether phylogenetic variation in growth responses to N enrichment across plant species is associated with variation in plant functional traits and nutrient co-limitation. Specifically, we tested three hypotheses: (1) phylogenetic groups differ in growth responses to N enrichment; (2) following the plant economics spectrum theory, species with more resource-acquisitive functional traits in ambient nutrient conditions exhibit greater growth responses to N enrichment (i.e., are more N-limited); and (3) P differentially limits biomass responses to N enrichment across species (i.e., P enrichment increases growth responses to N enrichment more in some species than others). To test these hypotheses, we applied factorial N and P fertilization treatments to the Tasmanian eucalypts in a controlled greenhouse environment. For each individual we quantified total biomass and three functional traits: specific root length (SRL), specific stem density (SSD), and specific leaf area (SLA). These traits are proxies for nutrient-uptake capacity, the ability to transport resources from roots to leaves, and photosynthetic activity, respectively (Pérez-Harguindeguy et al. 2013). We implemented a hierarchical Bayesian model, which allowed us to estimate multiple unknown parameters (nutrient limitation and the effects of functional traits and P on N limitation) while accounting for uncertainty within and across species. A benefit of this model is that different levels of the hierarchy (i.e., within and across species) inform one another, allowing for better estimation for groups with less information (e.g., species with fewer biomass and functional trait observations due to lower survivability). We expected to find strong differences in biomass responses to N between phylogenetic groups, greater biomass responses to N in species with more resource-acquisitive functional traits (greater SLA, lower SSD, and greater SRL) in control nutrient conditions, and different N by P interaction terms across species. Using a single model to examine the associations of phylogeny, functional traits, and P limitation with plant

N limitation, we provide a framework for predicting winners and losers in N deposition scenarios, and more important, test for novel linkages between past evolution and plant performance in global change scenarios.

Methods

Study system

Of the ≈ 700 described tree species within the genus *Eucalyptus* (Grattapaglia et al. 2012), 30 are native to the island state of Tasmania, Australia (Williams and Potts 1996, Gray 2008). According to both morphology (Brooker 2000) and genetic data (Steane et al. 2011), the Tasmanian eucalypts belong to two subgenera, *Symphyomyrtus* (470 total species in Australia and 17 in Tasmania) and *Eucalyptus* (108 total species in Australia and 13 in Tasmania; Grattapaglia et al. 2012). We chose to use the Tasmanian eucalypts in our study for three reasons. First, this set of eucalypts contains ecologically diverse and economically important species. The Tasmanian eucalypt species include growth forms from giant rainforest trees to stunted "mallee" shrubs, one of the most widely planted hardwood species across the globe (*E. globulus*), and several rare endemics (Williams and Potts 1996). Because the Tasmanian eucalypts within each subgenus have diverged across multiple soil nutrient, climatic, and topographical gradients and occupy habitats from coastal to sub-alpine regions and wet to dry sclerophyll woodlands (Williams and Potts 1996), we might expect genetic-based variation in resource-related functional traits within and among species. Second, empirical evidence has shown that species' performances and performance responses to global change are phylogenetically structured. For example, species within the *Symphyomyrtus* subgenus have faster growth rates than those within the *Eucalyptus* subgenus as seedlings and juveniles, which may explain why the former show greater survival and growth rates in a broader range of environments than the latter (Noble 1989, Anekonda et al. 1999). Subgenera also differ in responses to combined treatments of N fertilization and elevated CO_2, with species in subgenus *Symphyomyrtus* showing the most positive biomass responses to N and CO_2 (Senior et al. 2013, Wooliver et al. 2014). Third, evidence suggests that species differ in nutrient use strategies. *Eucalyptus* species have evolved in the relatively P-limited soils of Australia (Wild 1958), and Noble (1989) speculated that species within subgenus *Eucalyptus* have adapted to P limitation via greater dependence on mycorrhizal fungi than species within the subgenus *Symphyomyrtus*. Further, within Tasmania itself there are gradients in soil N and P, with species differing in mean soil N and P levels in their natural ranges (see Appendix S1: Fig. S1; data from Rossel et al. 2015), which may lead to variation in nutrient requirements for growth. Despite documented phylogenetic patterns in Tasmanian eucalypt growth responses to nitrogen enrichment, and indications that functional traits and nutrient use strategies vary across species, no study to date has addressed whether functional traits or differential co-limitation by P explain variation in biomass responses to N enrichment.

Phylogenetic reconstruction

We reconstructed the phylogeny of the Tasmanian eucalypts using a set of 3,881 parsimony informative Diversity Array Technology (DArT) markers (Jaccoud et al. 2001) and Metropolis-coupled Markov chain Monte Carlo in MrBayes v3.2 (Ronquist and Huelsenbeck 2003). We used DArT markers because they perform well at resolving phylogenetic relationships among *Eucalyptus* species (Jones et al. 2016). Genetic material for DArT markers was collected from individuals grown from seeds purchased from Forestry Tasmania (http://www.forestrytas.com.au/; see Appendix S1: Table S1 for information on seed collection zones), including the 23 species in the greenhouse experiment (described herein), plus four Tasmanian natives not included in the greenhouse experiment (*E. johnstonii*, *E. nitida*, *E. perriniana*, and *E. vernicosa*), and one non-native (*E. nitens*). The native Tasmanian species *E. archeri*, *E. morrisbyi*, and *E. nebulosa* were not included because seed was either not available or mislabeled. Full details of the phylogenetic reconstruction and the newick-formatted phylogeny are provided in Appendix S2 and Data S1, respectively.

After pruning out the species not used in our experiment, our phylogeny correctly placed species into their respective subgenera (Fig. 1a). Further, species within the subgenus *Symphyomyrtus* formed two phylogenetic lineages—(1) white gums and (2) alpine white, black, and yellow gums—while species within the subgenus *Eucalyptus* formed a third phylogenetic group composed of peppermints and ashes (Fig. 1a). We used these groupings to address whether phylogenetic groups differ in responses to N enrichment. Because the placement of *E. globulus* (the Tasmanian Blue Gum) into either of the gum lineages is uncertain (for this and other phylogenetic reconstructions, e.g., Steane et al. 2011, Senior et al. 2013, Jones et al. 2016), we excluded it from the lineage-level comparisons described herein.

Greenhouse experiment

We established a controlled greenhouse experiment at the University of Tennessee, Knoxville, in which we applied factorial treatments of N and P fertilization to 23 Tasmanian eucalypt species (12 in subgenus *Symphyomyrtus* and 11 in subgenus *Eucalyptus*), with five replicates per treatment/species combination arranged in a completely randomized design. We conducted the experiment in a greenhouse rather than a field setting to isolate genetic variation in plant functional traits and biomass production. Of the 1,150 plants (5 N fertilization levels × 2 P fertilization levels × 23 species × 5

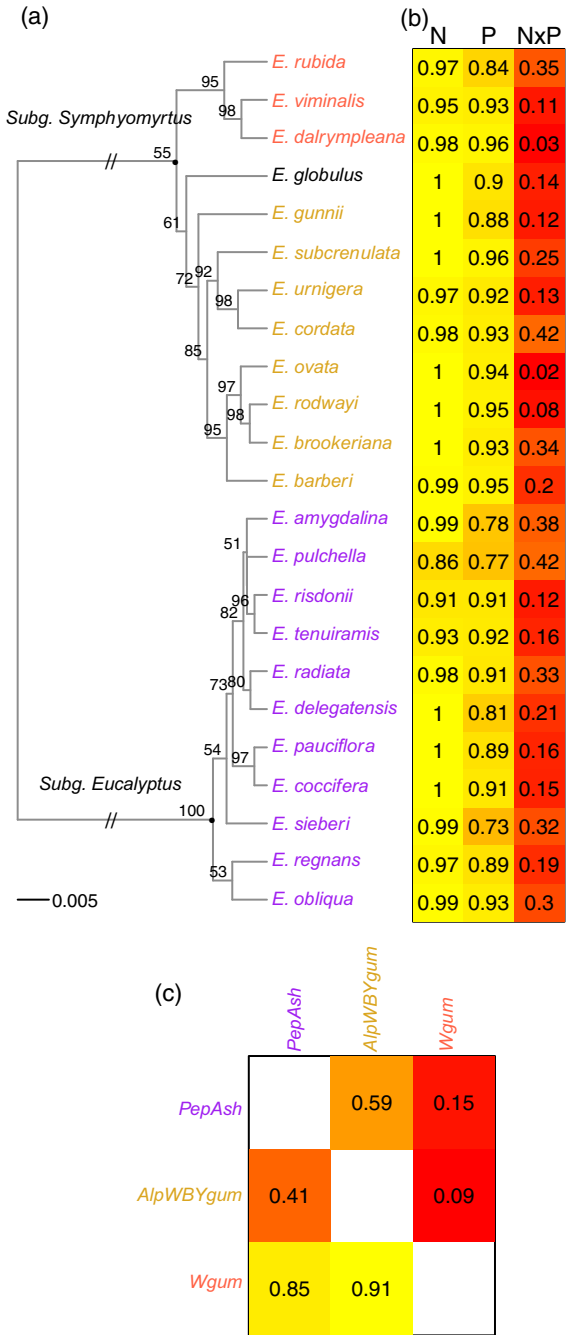

FIG. 1. (a) Phylogeny of 23 eucalypt species that are native to Tasmania, Australia. Percent posterior probabilities are shown at the top left of each node. To aid visualization, we have inserted a break in the branches connecting the subgenera (subg.), both of which constitute >98% of the phylogeny's arbitrary height of one (note the scale bar below the phylogeny). Species are colored by phylogenetic lineages, which include the white gums (red), alpine white, black, and yellow gums (gold), and peppermints and ashes (purple). (b) Percent of iterations in our Bayesian model in which nitrogen (N), phosphorus (P), and N × P coefficients for plant biomass were greater than zero for each species. Values are colored by a gradient from yellow (positive effect of nutrients) to red (negative effect of nutrients). (c) Percent of iterations in our Bayesian model in which N coefficients for biomass were greater for each column lineage compared to each row lineage. Lineages correspond to the phylogeny in (a). Values are colored from yellow (more positive responses to N) to red (more negative responses to N). [Color figure can be viewed at wileyonlinelibrary.com]

replicates), 931 survived; we collected SLA, SSD, SRL, and total biomass data from 722. Individuals were grown from seeds purchased from Forestry Tasmania (see Appendix S1: Table S1 for information on seed collection zones). For germination, we soaked seeds of each species in a petri dish between two pieces of Whatman number 1 filter papers (110 mm diameter; Whatman, Maidstone, Kent, UK) with 10 mL of 100 ppm gibberellic acid solution. After 24 h we spread the seeds over a 5 cm layer of moist non-fertilized potting soil (General Purpose Pro-Mix® BX, Premier Horticulture Inc.) and covered the seeds with another 0.5 cm layer of the potting soil. After 2 weeks of growth we transplanted 50 seedlings per species into separate 270-cm^3 pots containing the non-fertilized potting soil and initiated monthly N (urea) treatments at each of five rates (0, 1.2, 2.4, 5, and 10 $g \cdot m^{-2} \cdot yr^{-1}$) to 10 individuals of each species. Though exceeding the predicted global average rate of N deposition to forests by the end of this century (~0.9 g $N \cdot m^{-2} \cdot yr^{-1}$, Lamarque et al. 2005), these rates fall within the range of current N deposition rates across the globe, which reach up to 17 g $N \cdot m^{-2} \cdot yr^{-1}$ (Berendse et al. 1993). To determine whether species' responses to N are differently co-limited by P, we applied P (Triple Super Phosphate, Bonide Products Inc., Oriskany, New York, USA) at the rate of 1.2 $g \cdot m^{-2} \cdot yr^{-1}$ (a rate which relieves P limitation in Australian plantations; May et al. 2009) to five individuals of each species within each N application rate. After 5 months of growth we transplanted individuals into 2 L pots containing the non-fertilized potting soil to avoid root-binding. For the duration of the experiment (11 months) individuals were watered three times weekly to keep the soil near field capacity (moist but not saturated) and to prevent plant growth limitation by water. Daily temperatures in the greenhouse varied between 21 and 24°C. We acknowledge that soil microbial communities in this study came only from local greenhouse sources and thus were not representative of soil microbial communities in Tasmania. Although such communities—especially mycorrhizal communities of both arbuscular or ectomycorrhizal fungi that dually colonize eucalypts (Adams et al. 2006)—play major roles in plant responses to nutrient additions (Johnson 1993), testing the effects of soil microbial communities on eucalypt performance was outside the scope of this study.

Data collection

To quantify species' biomass responses to N and P and interspecific variation in functional traits, we measured SLA, SSD, SRL, and total biomass in December

2014 (after 11 months of nutrient treatments) for each individual in the greenhouse experiment following the sampling methods of Pérez-Harguindeguy et al. (2013). We measured SLA by averaging the area (cm^2) of two randomly selected, relatively young but fully expanded, outer canopy leaves on the terminal shoot per individual divided by their respective masses (g) after being oven-dried at 70°C for 72 h. We measured SSD by dividing the oven-dried mass (mg) of a 5–10 cm section of the main stem by its fresh volume (mm^3), excluding individuals with a main stem less than 5 cm long (91 of the 931 surviving individuals). We measured SRL by dividing the total length (cm) of the root system (scanned in a 20 × 15 cm tray and quantified using WinRhizo, Regent Instruments Inc., Quebec, Canada) by its oven-dried mass (g). For individuals with more roots than could be scanned without obscuring underlying roots (253 of the 931 surviving individuals), we randomly sampled and scanned a ~1.5 g (fresh weight) subsection to quantify SRL. A linear model suggested that SRL of individuals with whole root systems scanned was significantly greater than individuals with random root sections scanned, but this is to be expected as plants with lower biomass—those with whole root systems scanned—tend to have finer roots (Kalliokoski et al. 2010). For each individual, we summed the leaf mass used to calculate SLA, stem mass used to calculate SSD, oven-dried root biomass used to calculate SRL, and the remaining above- and belowground oven-dried biomass to obtain total biomass. Because species grew under the same conditions, we considered the functional traits of individuals receiving no additional N or P to represent genetic variation and thus species' placements along the resource-acquisitive to resource-conservative growth strategy spectrum. The dataset of SLA, SSD, SRL, and total biomass of all individuals in the experiment are provided in Data S1.

Analysis

To estimate species' biomass responses to N enrichment and whether these responses are associated with shared ancestry, specific suites of functional traits, and P limitation, we created a hierarchical Bayesian model. Here we provide a broad overview of the model's structure that is essential to understanding how we tested our hypotheses. Model specifications are provided in Appendix S3, with descriptions of data, index, and parameters descriptions listed in Appendix S3: Tables S1 and S2.

We modeled biomass and functional trait data of individuals for which all values were present (n_{obs} = 722 of the original 1,150 individuals), log-transforming biomass and functional traits before analysis to increase conformance to normality. For each individual (i) of each species (j), log-biomass ($y_i^{(j)}$) and log-functional traits ($\vec{w}_i^{(j)}$) were modeled as:

$$y_i^{(j)} \sim \text{Normal}(\vec{\beta}_j \boldsymbol{X}_i, \sigma_j^{[y]}) \quad (1)$$

$$\vec{w}_{i,\ell}^{(j)} \sim \text{Normal}(\vec{\phi}_{\ell,j} \boldsymbol{X}_i, \sigma_{\ell,j}^{[w]}) \quad (2)$$

$\vec{\beta}_j$ is a vector of biomass coefficients (intercept, response to N, response to P, N × P interaction) for species j and $\boldsymbol{X}$ is the corresponding matrix of predictors. Φ is a 3-dimensional array of coefficients that represent responses of each functional trait ℓ for each species j to $\boldsymbol{X}$. We hierarchically modeled species-level $\vec{\phi}_{\ell,j}$ coefficients around a central value ($\vec{\phi}_\ell^*$) so that parameter estimates would be partially pooled across species (Gelman and Hill 2007). The standard deviations (σ) were similarly modeled. To model the effect of functional traits on biomass responses to N, the β coefficients were estimated with a second-level regression.

$$E(\beta_{j,k}) = \gamma_k + \vec{\delta}_k \cdot \vec{\phi}_{j,k=1} \quad (3)$$

The expected value of $\beta_{j,k}$ is determined by the overall intercept for predictor k (γ_k), the species-level intercepts for each functional trait ($\vec{\phi}_{j,k=1}$), and the effect of $\phi_{\ell,j,1}$ on $\beta_{j,k}$ ($\delta_{k,\ell}$). The actual values of $\beta_{j,k}$were modeled with a matrix normal distribution that allowed for covariance structures: correlation among coefficients within species ($\Sigma^{[\beta]}$) and phylogenetic correlation among species ($\Sigma^{[\lambda]}$). A phylogenetic covariance matrix $\boldsymbol{V}$ was derived from our phylogeny and treated as a fixed parameter. Within the model, $\Sigma^{[\lambda]} = \boldsymbol{V}$, except the off-diagonal elements were scaled by λ (Pagel 1999), a parameter that accounts for phylogenetic covariance between biomass and functional traits and ranges from 0 (no phylogenetic covariance between biomass responses to N and traits) to 1 (covariance between biomass responses to N and traits matching phylogenetic distances among species).

The model was implemented using Stan (Carpenter et al. 2016), a system for Bayesian inference that uses Hamiltonian Monte Carlo to efficiently sample the posterior distribution (Hoffman and Gelman 2014), via the rstan package (v2.11.1; Stan Development Team 2016) in R (v3.3.0; R Foundation for Statistical Computing 2016). We ran three chains for 2,000 iterations each, with the first 1,000 used for adaptive warmup. We checked for convergence using the $\hat{R}$ statistic (Gelman and Hill 2007) and by examining trace plots. We examined model fit with posterior predictive checks. See Data S1 for the model code and R code used in model implementation and hypothesis testing (described below).

Hypothesis testing

We characterized the strength of parameters by calculating the percentage of posterior iterations in which the parameter is positive (or in specified cases, negative).

Post-hoc pairwise differences between species, lineages, or subgenera for a parameter were calculated as the percent of iterations in which each species, lineage, or subgenus had a greater value than each other species, lineage, or subgenus. For all parameters or comparison between parameters, we used the value of 90% to represent a strong effect or difference. Before testing our hypotheses, we examined $\vec{\beta}_N$, $\vec{\beta}_P$, and $\vec{\beta}_{NP}$ (the coefficients for species-level biomass responses to N, P, and N+P, respectively) to quantify nutrient limitation and co-limitation for each species. $\vec{\beta}_N$, $\vec{\beta}_P$, and $\vec{\beta}_{NP}$ coefficients that were strongly positive for a particular species would suggest N-limitation, P-limitation, and nutrient co-limitation, respectively, in that species. Alternatively, $\vec{\beta}_N$, $\vec{\beta}_P$, and $\vec{\beta}_{NP}$ coefficients that were strongly negative would suggest that plants suffer from non-optimal nutrient balance due to factors such as nutrient toxicity (Goyal and Huffaker 1984) or decreased benefit from mycorrhizal fungi (Johnson 1993). $\vec{\beta}_N$, $\vec{\beta}_P$, and $\vec{\beta}_{NP}$ coefficients that were neither strongly positive nor negative would suggest that plant growth is neither limited nor co-limited by N and P.

Hypothesis 1.—To test whether phylogenetic groups differ in growth responses to N, we averaged all iterations of $\vec{\beta}_N$ across species of each subgenus and across species of each lineage; we then performed pairwise comparisons for $\vec{\beta}_N$ between subgenera and lineages. Strong differences in responses to N would support our hypothesis that phylogenetic groups differ in growth responses to N.

Hypothesis 2.—To test whether more N-limited species are those with more resource-acquisitive functional traits, we examined the effects of $\vec{\phi}_{k=1}$ on β_N ($\vec{\delta}_{k=2}$, hereafter referred to as $\vec{\delta}_N$). $\delta_{N,\ell}$ can be interpreted as the change in $\vec{\beta}_N$ (i.e., the response of log-biomass to N) with each unit increase in the species-level average of log functional trait ℓ. Strongly positive (>90% iterations greater than zero) $\delta_{N,\ell}$ for SLA and SRL and negative (>90% iterations less than zero) $\delta_{N,\ell}$ for SSD would support this hypothesis. Alternatively, $\delta_{N,\ell}$ coefficients which are neither strongly positive nor negative would suggest that capacity for nutrient uptake from soils (indicated by SRL), capacity for nutrient transport from roots to leaves (indicated by SSD), and photosynthetic activity (indicated by SLA) do not influence N use capacity. If functional traits are phylogenetically conserved (e.g., greater on average in one lineage compared to another), our model would identify functional trait effects on biomass responses to N enrichment as effects of phylogeny.

Hypothesis 3.—To test whether P differentially limits species' responses to N, we performed pairwise comparisons of $\vec{\beta}_{NP}$ among species. Strong differences would support the hypothesis that P differentially co-limits species' responses to N enrichment.

Results

Nutrient limitation and co-limitation across species

Overall, most eucalypt species responded positively to increasing N addition (Figs. 1b and 2). The estimated responses to N enrichment were strongly positive for 22 out of 23 species (Fig. 1b). When back-transformed, mean responses ranged three-fold: from 6.21 (*E. pulchella*) to 16.87 (*E. delegatensis*) percent increases in biomass with each g $N \cdot m^{-2} \cdot yr^{-1}$ added. Mean percent increases in response to P enrichment were larger than those in response to N enrichment: back-transformed mean responses ranged two-fold from 20.80 (*E. sieberi*) to 40.07 (*E. coccifera*) percent increases in biomass with 1.2 g $P \cdot m^{-2} \cdot yr^{-1}$ added (compare biomass at low vs. high P at 0 g $N \cdot m^{-2} \cdot yr^{-1}$ in Fig. 2). However, a fewer number (only 15) of the 23 species showed strong positive responses to P enrichment than to N enrichment (Fig. 1b). In contrast to expectations, P enrichment did not strongly affect species' responses to N enrichment; the percent of iterations in which the N × P interaction term was greater than zero did not reach 90% for any species (Fig. 1b; compare slopes of biomass across N between low and high P in Fig. 2). Rather, for three species, *E. dalrympleana*, *E. ovata*, and *E. rodwayi*, P addition strongly *reduced* biomass responses to N enrichment (Fig. 1b). For example, the mean responses of *E. dalrympleana* to N enrichment without and with P addition were 7.48 and 1.30% increases with each unit N, respectively.

Nitrogen limitation across phylogenetic groups

In support of our first hypothesis, pairwise comparisons of lineage responses to N revealed that for 91% of iterations, the alpine white, black, and yellow gum lineage responded more to N than the white gum lineage (Fig. 1c). When back-transformed, mean percent increases in biomass with each g $N \cdot m^{-2} \cdot yr^{-1}$ added were 11.80 (alpine white, black, and yellow gums), 11.21 (peppermints and ashes), and 8.01 (white gums). The pairwise comparison between subgenera revealed no strong difference in subgenus responses to N; for 61% of iterations, species within subgenus *Eucalyptus* responded more strongly to N than species within subgenus *Symphyomyrtus*.

Associations between functional traits and nitrogen limitation

Species' mean SLA intercepts estimated from the Bayesian model varied from 101 cm^2/g (*E. gunnii*) to 181 cm^2/g (*E. pulchella*); mean SSD intercepts varied from 0.428 mg/mm^3 (*E. sieberi*) to 0.477 mg/mm^3 (*E. barberi*); and mean SRL intercepts varied from 5,487 cm/g (*E. brookeriana*) to 8,548 cm/g (*E. delegatensis*) (Fig. 3). However, in contrast to our second hypothesis, we found no strong associations between species' functional traits and biomass responses to N

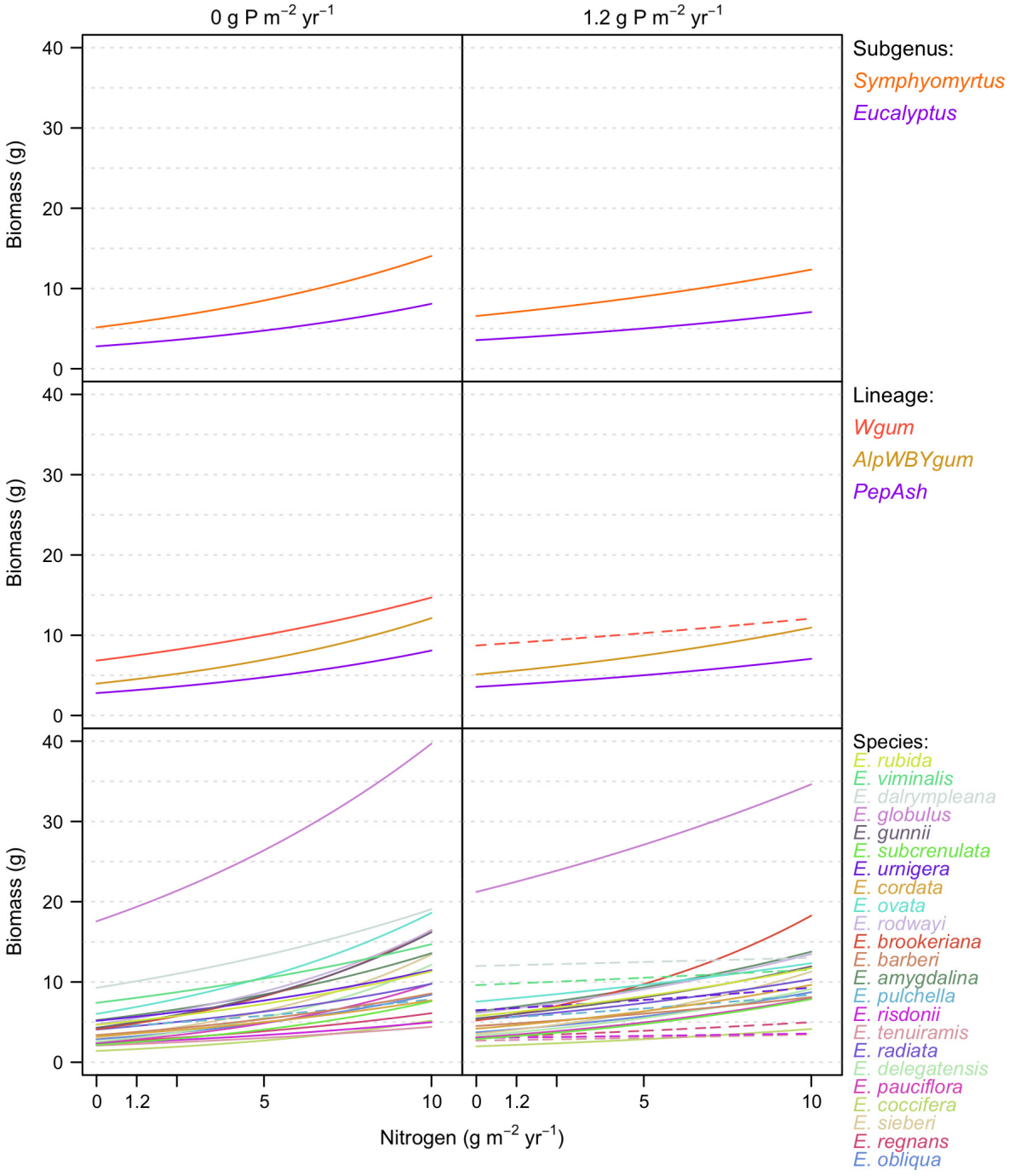

FIG. 2. Nitrogen (N) limitation and co-limitation by phosphorus (P) across 23 eucalypt tree species that are native to Tasmania, Australia. Response curves represent changes in biomass across a gradient of N (0–10 g·m^{-2}·yr^{-1}) with and without P enrichment (right and left, respectively). Responses are shown at the subgenus (top), lineage (middle), and species (bottom) levels. Lineages include peppermints and ashes (PepAsh), alpine white, black, and yellow gums (AlpWBYgum), and white gums (Wgum). Solid and dashed lines represent plant responses to N that were strong (>90% of posterior iterations from our Bayesian model greater than 0) and weak (<90% of posterior iterations from our Bayesian model greater than 0). [Color figure can be viewed at wileyonlinelibrary.com]

enrichment (Appendix S4: Fig. S1). This was after we corrected for covariation between species' functional traits and species' biomass coefficients due to phylogenetic non-independence (mean $\lambda = 0.203$ with 95% credibility interval between 0.017 and 0.5570). The mean effect of SLA was −0.12, with 20% of iterations estimating effects *greater* than 0; the mean effect of SSD was −0.45, with 77% of iterations estimating

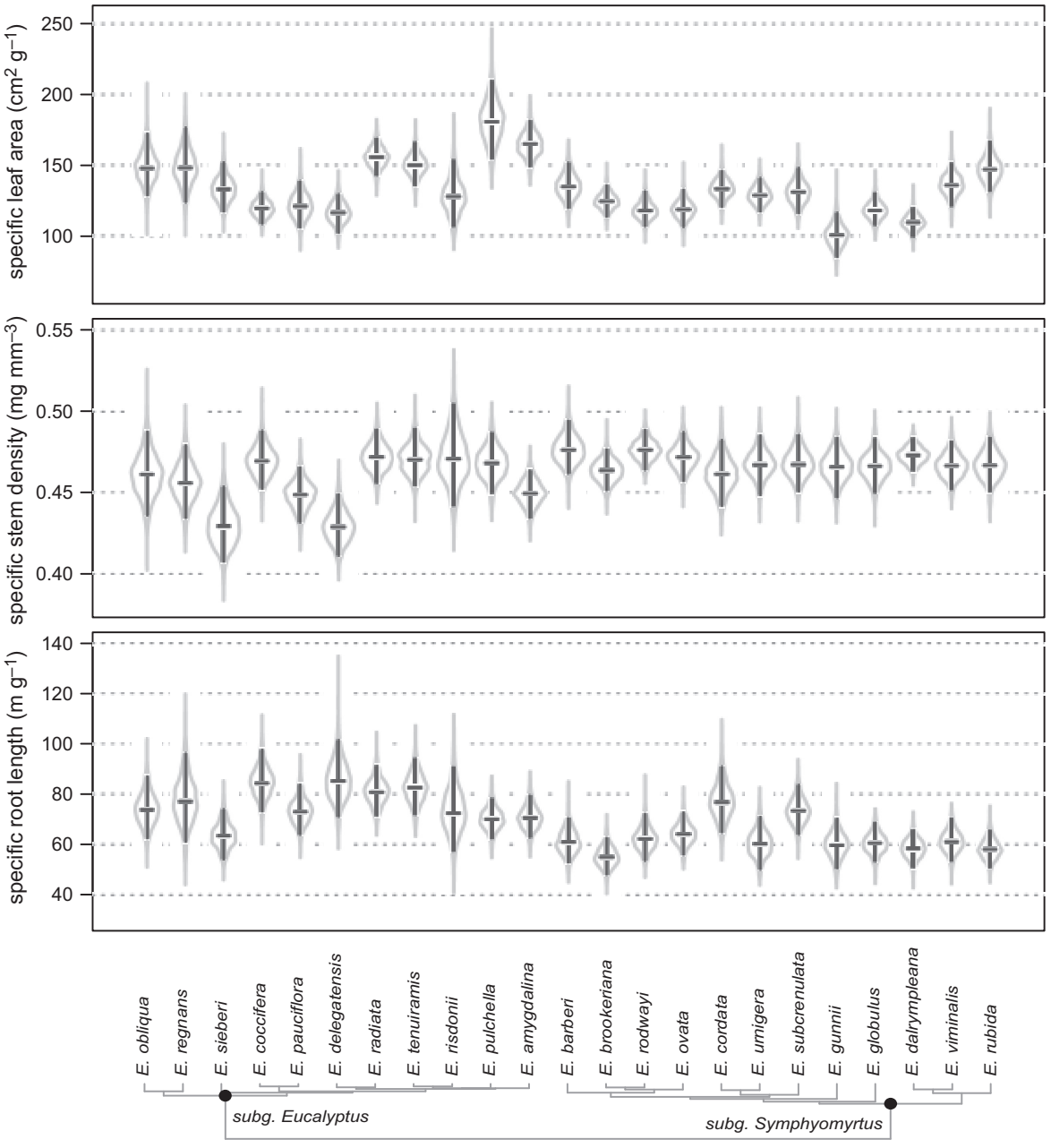

FIG. 3. Species-level variation in functional traits across Tasmanian eucalypts. Traits include specific leaf area (SLA; top panel), specific stem density (SSD; middle panel), and specific root length (SRL; bottom panel) in standard potting mix with no additional nutrients (i.e., the trait intercepts in our Bayesian model). Species are arranged according to their phylogeny (shown below). Vertical black lines represent 95% credibility intervals.

effects *less* than 0; and the mean effect of SRL was −0.01, with 47% of iterations estimating effects *greater* than 0 (Appendix S4: Fig. S1).

Nutrient co-limitation across species

In contrast to our third hypothesis, pairwise comparisons of N × P coefficients did not reveal strong differences between species. That is, for no pair of species did N × P coefficients of one species exceed those of another species for more than 90% of iterations (Appendix S4: Fig. S2).

DISCUSSION

We found that phylogeny is an important tool for predicting plant responses to N enrichment. Specifically, we found overall that Tasmanian eucalypt species are

N-limited (Figs. 1b and 2) but vary three-fold in their responses to N enrichment, corroborating evidence that continued anthropogenic increases in soil N will lead to greater community productivity (Kulmatiski et al. 2007, LeBauer and Treseder 2008) and lower species richness through competitive exclusion of less nitrophilic species (Bobbink et al. 2010, De Schrijver et al. 2011). Post-hoc pairwise comparisons among phylogenetic lineages showed that on average some groups have stronger responses to N addition than others (Fig. 1c), suggesting that phylogeny can be used to predict winners and losers in future N deposition scenarios. We found that although species vary in functional traits that are commonly used to represent resource-use strategies (Fig. 3), these traits do not underlie variation in responses to N enrichment (Appendix S4: Fig. S1). This finding suggests that the plant economics spectrum (Grime 1977, Chapin 1980, Wright et al. 2004, Craine 2009, Reich 2014) will not be a useful tool to predict winners (more nitrophilic species) and losers (less nitrophilic species) for Tasmanian eucalypt communities, and perhaps other communities, under increased rates of N deposition. Finally, we found that P does not differentially limit species' responses to N enrichment (Appendix S4: Fig. S2). This indicates that, although Tasmanian eucalypt species have evolved in P-limited soils (Wild 1958), their solutions for dealing with limitation by P (Cleland and Harpole 2010) do not generate strong variation in how P affects seedling performances in future N deposition scenarios.

Effects of phosphorus on nitrogen limitation

In accordance with evidence that productivity can be limited by N or P (Elser et al. 2007, Harpole et al. 2011), we found overall that Tasmanian eucalypt species respond positively to N or P enrichment. Thus, N and P limitation may play a role in governing eucalypt seedling productivity and increasing rates of anthropogenic N enrichment of soils should increase productivity in eucalypt forests. However, in contrast to evidence that plant growth can be co-limited by soil N and P (Elser et al. 2007, Harpole et al. 2011), we found that P addition does not strongly increase responses of Tasmanian eucalypts to N enrichment overall (Figs. 1b and 2). In fact, responses of three species to N may be reduced by P enrichment (Fig. 1b), a sub-additive nutrient response (i.e., a change in biomass in response to both N and P enrichment that is less than the sum of the responses to each nutrient added independently) that previous work suggests is not uncommon in community-level productivity (Harpole et al. 2011).

Sub-additive responses to N and P fertilization in eucalypts could stem from several factors which may not be mutually exclusive. One such factor might be the interaction between plants and mycorrhizal fungi, on which eucalypts have been shown to depend strongly for nutrient acquisition (e.g., Bougher et al. 1990). Eucalypts are known plant genera that form dual associations with both arbuscular mycorrhizal (AM) and ectomycorrhizal (EM) fungi, generally forming associations predominantly with AM fungi as seedlings and switching to EM fungal associations as they mature (Adams et al. 2006). The timing of this switch varies across species (dos Santos et al. 2001) and may be an important factor driving variation in species' responses to nutrient enrichment. Though we have pursued subsequent fertilization experiments that include live soil inocula collected from trees in Tasmania to understand how native soil microbiomes influence eucalypt responses to N (which we plan to publish in separate manuscript), inoculating the plants with mycorrhizal fungi from their natural ranges in Tasmania was not possible for this study. Rather, spores of 'weedy' mycorrhizal fungi that are not typically representative of natural communities are common in greenhouse settings. Species whose responses to N appear to be reduced by P enrichment may have shown such responses due to a shift in mycorrhizal fungal communities from mutualistic at low soil P levels to parasitic at high soil P levels (Johnson 1993). Alternatively, the overall growth of plants in this study may have not been compatible with (and potentially not as much affected by) foreign mycorrhizal fungal communities, as shown in previous inoculation experiments with *Eucalyptus* (Malajczuk et al. 1984, Burgess et al. 1994), due to co-evolution with unique mycorrhizal fungal communities during long periods of geographic isolation in Australia (Bougher 1995). The impact of mycorrhizal fungi (especially those that commonly associate with Tasmanian eucalypts) on resource uptake was beyond the scope of this experiment, but future work to inoculate eucalypts with native soil fungi, score mycorrhizal colonization, and sequence soils for fungal DNA in fertilization experiments could provide insight into whether soil fungi mediate the responses of Tasmanian eucalypt seedling responses to soil nutrient enrichment. Alternatively, high levels of N and P may have led to nutrient toxicity (Goyal and Huffaker 1984), where uptake of excess nutrients becomes metabolically and physiologically costly, or to increased photosynthesis, transpiration losses, and soil moisture loss, thereby exacerbating limitation by soil moisture (W. S. Harpole, *personal communication*).

Sub-additive eucalypt responses to N and P fertilization have important implications for our understanding of plant responses to nutrients in both agricultural and natural settings. For example, *E. globulus*, because it is fast-growing and has large flowers that facilitate breeding studies, is one of the most prominent species for pulpwood production across the globe (Williams and Potts 1996). We identified a moderately reduced response to N enrichment with the addition of P fertilizer (Figs. 1b and 2), indicating that the N + P fertilizers used in agricultural trials (reviewed by May et al. 2009) may lead to less production than would fertilizers including N or P alone. Although our results suggest that P limitation may be an important factor governing responses to anthropogenic increases in soil N for some

eucalypt species, co-limitation by P may not drive differential responses to N in these scenarios.

Associations between functional traits and nitrogen limitation

The plant economics spectrum theory argues that a plant's evolved capacity for resource use reflects an evolutionary tradeoff between resource-acquisitive and resource-conservative growth strategies (Grime 1977, Chapin 1980, Wright et al. 2004, Craine 2009, Reich 2014). Recent work has demonstrated that functional traits that predict where species fall along the resource-acquisitive to resource-conservative spectrum contribute to plant responses to global change. For example, plant species with more resource-acquisitive functional traits (including high photosynthetic capacity and short leaf lifespan) at ambient temperatures gain more biomass in response to warming (Gornish and Prather 2014). The eucalypt species in this study show strong genetic variation in their functional traits (Fig. 3), suggesting that they occupy different places along the resource-acquisitive to resource-conservative spectrum. However, we find that Tasmanian eucalypts not only represent a small portion of phylogenetic variation but also span only a small portion of the range of functional traits observed across terrestrial plant species (from 9 to 1137 cm^2/g for SLA, from nearly 0 to 1.331 mg/mm^3 for SSD, and from 608 to 75,681 cm/g for SRL; Kattge et al. 2011). Such small variation in functional traits may explain why our model revealed no strong associations between resource-use traits and growth responses to N enrichment (Appendix S4: Fig. S1), nor between resource-use traits and biomass production in control nutrient conditions, as might also be predicted by the plant economics spectrum theory. Given the global variation in terrestrial plant functional traits (Kattge et al. 2011) and responses to N enrichment (Wooliver et al. 2016), the hierarchical Bayesian model implemented in this study should recover any significant associations that truly exist between functional traits and growth responses to N enrichment should it be broadened to an analysis of plant species across the globe.

Phylogenetic variation in N limitation

Our results corroborate previous evidence showing that phylogenies are important for explaining variation in plant species' growth responses to N enrichment (Wooliver et al. 2016). Specifically, we show that species within the white gum lineage respond less positively to N than do species within the other two lineages (Figs. 1b and 2). Such a pattern indicates that species within the white gum lineage may share functional traits that limit the ability to use excess N for growth compared to species within the other two lineages. Although our analysis revealed that responses to N are not associated with leaf, stem, or root functional traits, additional post-hoc pairwise comparisons showed that species within the white gum lineage have consistently lower SRL (indicative of a more resource-conservative growth strategy; Reich 2014) in control nutrient conditions than species within the other two lineages (Appendix S4: Fig. S3) which we note is likely not an artifact of a lower proportion of individuals whose whole vs. subsampled root system was scanned to calculate SRL (Appendix S4: Fig. S4). This pattern may not have been detected in the Bayesian model because the model controls for phylogenetic covariance between functional traits and biomass responses to N enrichment. That is, our model is constructed so that effects of functional traits on biomass responses to N enrichment are interpreted as effects of phylogeny should those traits be phylogenetically conserved. We also note that lower SRL may contribute to greater growth of white and blue gums overall, notably the Tasmanian blue gum *E. globulus* (Fig. 2), and may underlie the greater overall growth rates of species within the subgenus *Symphyomyrtus* than those within the *Eucalyptus* subgenus seen here and in previous studies (Noble 1989, Anekonda et al. 1999). Together these results suggest that traits determining species' positions along a root economics spectrum are phylogenetically based and that this phylogenetic basis to trait variation could underlie species responses to anthropogenic N enrichment of soils.

Additional post-hoc pairwise comparisons revealed that there are also strong species-level differences in responses to N enrichment (Appendix S4: Fig. S5). For example, the response of *E. brookeriana* (black gum) to N enrichment was greater than responses of *E. viminalis* (white gum), *E. dalrympleana* (white gum), *E. globulus* (blue gum), and *E. urnigera* (alpine white gum) for 90% or more of iterations in the model. However, long-term N fertilization trials in natural eucalypt communities are required to determine whether nitrophilic eucalypt species will competitively exclude other, less nitrophilic species under future N deposition scenarios (Bobbink et al. 2010). These data overall suggest that who will win and who will lose in these scenarios, as highlighted earlier, is strongly governed by shared ancestry. Thus, phylogenies represent powerful tools for predicting shifts in plant community composition as rates of N deposition continue to rise. However, future work is needed to pinpoint the proximate determinants, such as interactions with soil microbes or plasticity in functional traits such as SRL, that underlie phylogenetic patterns in plant performance responses to N deposition.

Conclusions

We have explored variation in species' responses to N enrichment for seedlings of 23 eucalypt species that are native to Tasmania, Australia, and whether this variation is explained by phylogeny, specific functional traits, or co-limitation by P using hierarchical Bayesian modeling. While these outcomes are not mutually exclusive, we found that the growth of a majority of the eucalypt

species is strongly limited by N and shared evolutionary history explains variation in the degree of N limitation. However, results show that seedling responses to N are not associated with co-limitation by P, nor has the evolution of functional traits guided variation in responses to N in this experiment (although we find a phylogenetic pattern in root traits similar to that of responses to N). Because Tasmanian eucalypt species represent only a small portion of functional variation across terrestrial plant species, we suggest that the analysis we implemented may identify associations of growth responses to N enrichment with P limitation and functional variation across species that exhibit greater variation in N use strategies. However, our results indicate that even within groups of closely related, co-occurring species, phylogeny will likely be a powerful tool for predicting winners and losers in N deposition scenarios. Continued work to identify ecological mechanisms that underlie phylogenetic patterns in plant responses should provide insight into why these patterns exist and how they will play out across landscapes under global change.

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